ON THE STERILIZATION OF *DROSOPHILA* BY HIGH TEMPERATURE.

WILLIAM C. YOUNG AND HAROLD H. PLOUGH,

FROM THE BIOLOGICAL LABORATORY OF AMHERST COLLEGE, AMHERST.

Breeding experiments with *Drosophila melanogaster* at high temperatures have demonstrated that at a point 5° C. to 9° C. above the normal optimum of 24° C. no offspring are produced after one generation. This fact was first noted by Northrop (1) for a single stock. Plough and Strauss (2) showed that the exact temperature at which complete sterilization occurred varied with different stocks, but was approximately constant for any particular one. In all cases a temperature was found which produced complete sterilization after an exposure of four days or more, even though adult flies continued to live for some time at this temperature, and newly laid eggs developed, pupated and went through metamorphosis normally enough. The additional fact was noted by all these workers that flies thus sterilized would usually recover normal fertility if they were replaced for a day or two at a temperature of 24 degrees. Northrop stated however: "If after they have emerged from the pupæ they are left longer than ten days at the higher temperature the injury becomes permanent and they are no longer able to produce eggs capable of development at any temperature."

Attempts to explain this sterility in terms of effects on the germ cells have not been entirely successful. Northrop stated, though without crucial evidence, that "Imagos raised and kept permanently at a temperature of 30 degrees, are unable to produce eggs capable of development at this temperature." Plough and Strauss demonstrated that this could not be the correct explanation by crossing both males and females hatched at 31 degrees to females and males respectively bred at 25 degrees, carrying the crosses at 31 degrees. Most of the 31 degree females mated to normal males gave offspring at 31 degrees, and about half of the 31 degree males likewise. The actual figures summarized from Plough and Strauss, Table II., are:

	Fertile Cultures.	Sterile Cultures.
31° ♀ × 25° ♂	13	2
25° ♀ × 31° ♂	6	5

In spite of the excess in the number of fertile cultures from females this result was taken to indicate that "neither the eggs nor sperm are injured by exposure to 31° C." Cytological examination showed that apparently normal eggs and sperm were present in flies sterilized by heat, and copulation was observed between these flies at the high temperature. Plough and Strauss thus summed up the matter: "The reason for the failure to produce offspring after the first generation seems to lie either in a failure of the sperm to reach the eggs,—that is unsuccessful copulation—or in the failure of the fertilization process itself."

Further investigation of the question was delayed until the winter of 1924–5 when one of us—Young—was making a series of tests designed to determine whether strains of flies showing differing degrees of toleration of high temperature could be isolated from a single mass culture of a wild stock. The results of this series of tests will be reported elsewhere, but the experiments offered an excellent opportunity to settle the question raised above.

An examination of the Plough and Strauss data quoted above shows only that eggs and sperm are not injured by exposure to 31 degrees in every case. Sterile cultures did occur, and a comparison of the relative numbers suggests, in addition, that males are more likely to be sterilized than females. Since the 31 degree flies used were not actually tested and shown to be sterile at 31 degrees, but were taken from susceptible stocks (*i.e.*, stocks which ordinarily failed to reproduce at 31 degrees), it is possible that some of the flies which gave offspring in crosses might have been fertile even if they had been mated inter se at 31 degrees. For these reasons we returned to the question of the possible injury to the germ cells of the flies by high temperature, and made more extended breeding tests, and more careful cytological examination of the gonads from flies sterile at 31 degrees. Most of the breeding tests were made by Young, while the remainder together with the cytological study were made by Plough.

Experiments.

The *Drosophila* stocks used for the tests were for the most part taken from a wild stock culture collected at Canton, Ohio, in September 1924, but for certain of the experiments other stocks were used as indicated. They showed a varying degree of toleration of high temperature as recorded by Plough and Strauss, but even though the critical temperature was different for different stocks each could be rendered sterile at some particular temperature. Even when a culture showed sterility it was observed that copulation between the flies takes place, and that eggs were laid in numbers roughly equal to those from similar flies at normal temperature. Since these eggs appear to be normal and since virgin females are known to lay eggs under normal temperature conditions, the conclusion is again suggested that the sterility at high temperature is caused by some failure to function on the part of the males.

Further data similar to those of Plough and Strauss were first collected. A series of lines of Canton stock were being run at 31 degrees. Since this temperature is close to the limit at which this stock can be bred, it was found that several strains usually showed sterility in each generation. There was a high degree of constancy in the reaction to temperature of these strains, some failing regularly after one generation, others running along for several generations normally. Matings were made at 31 degrees between both males and females from a number of these lines and flies of the opposite sex from the same strain carried continuously at 24 degrees. Normal flies of this stock are always fertile for at least one generation at 31 degrees. If a culture of the 31 degree stock showed sterility, then it might be supposed that the mating of sibs of this strain to the 24 degree stocks would indicate whether one sex rather than the other was responsible for the failure. The cultures in all cases contained five pairs of flies each, and a summary of the tests is given in Table I.

A comparison of the totals in this table emphasizes the suggestion of the summaries of the similar tests of Plough and Strauss, namely that most of the males from a culture at 31 degrees are sterile, while most of the females are fertile. This

TABLE I.

TESTS RUN AT 31 DEGREES.

Controls (Sibs of Tested Flies at 31°).	♂ Hatched at 31° × ♀ at 24°.		♀ from 31° × ♂ from 25°.	
	Sterile.	Fertile.	Sterile.	Fertile.
Fertile—16	8	8	0	16
Sterile—12	11	1	6	5
Total Cultures	19	9	6	21

becomes still more significant when the figures are examined in comparison with the corresponding controls. Where these were fertile, all of the females were fertile, yet one half of the males were sterile. Apparently the males of a culture begin to be sterilized by heat, before there is any effect on the females whatever. When the controls were sterile, in only one out of twelve cultures did the males show fertility, while about half of the females are affected and half are still fertile. It would appear that high temperature causes at least partial sterilization of the males at a point where the females are largely unaffected. Eventually at least some of the females are sterilized. It was noticeable that in the strains showing a high degree of tolerance for 31 degrees, the males only showed sterility.

In order to establish these facts beyond question it seemed wise to run a more extended series of matings to normal stock using cultures each of which was actually known to be sterile at high temperature. A series of cultures of flies of different strains was placed in the incubator at 32 degrees. This is sufficiently high to cause sterility in nearly all strains after the first generation, and in every one of the cases tabulated this was the result. Each fly was then mated to another of the opposite sex which had been hatched at the optimum temperature of 24 degrees, and all were replaced at 32 degrees. The results of these tests are given in Table II.

The totals show that from cultures which gave no offspring at high temperature, 96 per cent. of the males were actually sterile, while only 50 per cent. of the females were affected. More detailed analysis makes plain the fact already demonstrated by

TABLE II.

FLIES STERILE AT 32°. REMATED AND CONTINUED AT 32°.

The numbers given refer to individual flies.

Stocks.	♂ from 32° × ♀ from 25°.		♀ from 32° × ♂ from 25°.	
	Sterile.	Fertile.	Sterile.	Fertile.
(1) Porto Rico	12	0	0	16
(2) Canton	3	0	0	3
(3) Amherst	8	2	8	4
(4) Prague	12	0	6	3
(5) Brown	6	0	6	0
(6) Spineless	6	0	6	0
Totals	47	2	26	26
%	96%	04%	50%	50%

Plough and Strauss, by Strauss (3), and by the unpublished work of Young mentioned above, that stocks show differences in their reactions to high temperature, some being much more tolerant than others. In the most tolerant stocks—Porto Rico and Canton—there is a clear differential effect such that the males are rendered completely sterile while the females are unaffected. In less tolerant stocks like the Prague and Amherst cultures the females show some effect, though it is not so great as in the case of the males. In mutant stocks like spineless and brown there is much less resistance and both sexes are rendered completely sterile. From these and other data there is evidence that at a temperature somewhat lower than 32 degrees even these stocks show the same differential sterilization of the males without any effect on the females.

Turning now to experiments designed to test the length of time required for flies sterile at 31 degrees to recover fertility when returned to 24 degrees, we find this selective effect of high temperature on the male flies again clearly demonstrated. This recovery of fertility—first noted by Northrop as indicated above—was shown by removing cultures from the 31 degree incubator at intervals and placing them in new bottles at 24 degrees. Controls were furnished by cultures of normal flies bred continuously at 24 degrees, and also by females from the sterile 31 degree cultures mated to normal males. In all cases the bottles

contained five pairs of flies each, so that individual differences are largely eliminated from the result. The time of the appearance of the first eggs and first larvæ are noted in each case, and the data are tabulated in Table III.

Table III.

Canton Stock Cultures Sterile at 31°, Removed and Placed at 24°.

Description.	No. Days at 31° after Hatching.	No. Cultures.	Average No. Days before Eggs Appeared.	Average No. Days before Larvæ Appeared.	No. Cultures Failed to Recover.
Flies bred at 24° continuously.....	0	17	1.5	2.5	—
Females from sterile cultures × males bred at 24°.....	10 (♀ only)	7	1.5	3	0
Sterile 31° cultures.....	2	4	1.5	7.5	0
" " "	6	8	1.5	7.5	1 (12½%)
" " "	10	6	1.5	9	1 (16.6%)
" " "	13	13	1.5	9	5 (38.4%)

For the cultures recorded in Table III. the date of the first adult flies was recorded also, but is not noted in the table since the average number of days between the appearance of larvæ and the emergence of imagos was the same in all cases—about six days. The second line in the table affirms the result given in Tables I. and II., namely that females are not affected by a temperature of 31 degrees and their eggs develop as soon as fertilized. The remainder of the table therefore indicates the effect of the high temperature on the male flies. The females begin to lay eggs at once, but they do not lay eggs which develop until a period of seven to nine days has elapsed. The time when fertile eggs begin to be laid bears only a slight relation to the length of exposure to high temperature after hatching, but the last column showing the number of cultures which failed to recover, indicates that more males never regain fertility after a longer exposure.

The experimental data apparently demonstrate that high temperature has a differential action on the germ cells of *Drosophila* such that males are rendered completely sterile at a point at which females are uninjured. This effect is permanent as long as the temperature is maintained, but male flies will recover normal fertility if returned to the optimum temperature for a variable period. Longer exposures, however, tend to greatly increase the number of male flies which are permanently sterilized. Apparently raising the temperature still higher eventually sterilizes the females as well, and finally kills the flies.

Cytological Observations.

Sections of testes and of ovaries from flies which failed to produce offspring at 31 degrees were made, and compared with similar sections from normal flies of the same age. In addition the spermathecæ and ventral receptacles of females of the two sorts were examined both whole in Ringer's solution and in stained sections.

Study of these preparations adds very little to the facts already shown by experiment, though it confirms these. In none of the sections is it possible to discover any difference in the cytological picture between normal ovaries and ovaries of females from sterile cultures, except that the latter appear to be smaller, with fewer eggs at similar stages. Sections of the widened end of the testis just about at the point where it joins the vas deferens were studied in a sterile male and in a normal male respectively. The testes were taken from flies one group of which had been hatched and kept at 31 degrees for twelve days and the other at 24 degrees for the same time. The spermatozoa in the latter completely fill and distend the surrounding membrane. The same region in a sterile male is partially collapsed, and contains only a few scattered sperm. In other sections even these are absent. In portions of the testis slightly further up one can see a few cells which are probably spermatocytes together with some spermatozoa. At about the same place in testes from sterile flies only masses of spermatozoa appear. These seem to be shrunken away from the wall in masses and appear to be undergoing degeneration for neither their size nor arrangement is nor-

mal. In other sections from sterile males degeneration is also suggested for the sperms are decidedly reduced in number, have lost their orderly arrangement in masses in the testis, and seem shrunken in compact irregular aggregations. Apparently a few pass down into the vas deferens but these are probably not functional.

Ventral receptacles of females from sterile and fertile cultures respectively were also examined. Actual dissection of such flies in Ringer's solution shows clearly that spermatozoa are present in the receptacles of normal flies, but they can not be found in the females from sterile cultures. The sections simply confirm these findings. Masses of sperm can be seen clearly to fill the lumen of the receptacles of flies from normal cultures while ducts of flies from sterile cultures are empty. These observations prove that even though the male and female flies of sterile cultures copulate no transference of sperm to the receptacles of the females takes place.

Beyond these facts cytological study reveals little. The testes of sterile flies are smaller on the average, and there is some suggestion of disorganization in the cells in the lower portion, but this is not constant. However it has been shown above that exposure of the adult males to 31 degrees for ten days or more produces sterility from which a certain number do not recover when they are placed at 24 degrees. If only the spermatozoa are affected, it is hard to see why this should happen. The data showing the recovery of the male flies suggests a progressive sterilizing effect beginning with the sperms and eventually reaching cells at earlier stages. Only when the latter occurred would the male fail to recover fertility when placed at 24 degrees.

It is interesting that one pair of testes greatly reduced in size was found in a sterile male and sectioned separately. Both testes appear to have been but empty shells for neither spermatozoa nor any clear germinal tissue whatever were present. This may have been a degenerative change produced by high temperature, but such testes occasionally appear in normal stock. None did appear among the controls examined, however.

The cytological observations therefore confirm the breeding

tests by showing that a temperature of 31 degrees sterilizes male flies, with little or no effect on the females. The sterility appears to be caused by some degenerative effect on the spermatozoa, such that all become non-motile, and eventually may be killed. Recovery apparently consists in the formation of spermatozoa anew from cells in the growth period or earlier.

Discussion.

These facts are of especial interest when considered with others recently recorded. Cells of the male germ line are here shown to be much more sensitive than eggs or other tissues to a rise of 6 or 7 degrees in a cold-blooded animal, the fruit fly. C. R. Moore (4) has given similar evidence of the sensitiveness of the male germinal epithelium in warm blooded animals in his extensive studies on experimental cryptorchidism in mammals. He finds that when testes are exposed to the higher temperature of the peritoneal cavity rather than the scrotum there follows a rapid and progressive degeneration of the germinal cells. Such testes show progressive recovery when returned to their normal position. Bluhm (5) has given interesting evidence of the greater sensitivity of spermatozoa of mice to drugs (alcohol, caffein, etc.) since a portion of one class of spermatozoa, the female producing, become non-functional. Further the recent tests of Mavor and DeForest (6) on the sensitivity of eggs and sperm of *Arbacia* to X-rays show a much greater sterilizing effect on the sperms at all doses. Evidently the male germ cells are much more susceptible than eggs or other cells of the animal body to external influences.

Summary.

1. High temperature has a differential effect on the germ cells of *Drosophila* such that males may be rendered completely sterile at a point at which females are still completely fertile.

2. This effect on the males is permanent as long as this temperature is maintained, but most males will recover normal fertility after being returned to the optimum temperature.

3. Exposures to 31° C. of over ten days tend to increase greatly the number of flies which are permanently sterilized.

4. Examination of the testes of sterile males shows that high

temperature causes loss of motility of the sperm, with progressive aggregation and degeneration. Few or no sperms are found in the lower end of the testis and the vas deferens.

5. Although such males copulate with females at high temperature, no sperms pass into the ventral receptacles of the females.

LITERATURE CITED.

1. **Northrop, J. H.**
'19–'20 J. Gen. Physiol., II, 313.
2. **Plough, H. H., and Strauss, M. B.**
'23–'24 J. Gen. Physiol., VI, 167.
3. **Strauss, M. B.**
'25 Am. Nat., LIX., July–August.
4. **Moore, C. R.**
'24–'25 Am. J. of Anat., 34, 269, 337.
5. **Bluhm, A.**
'24 Arch. für Rass. u. Gesellschafts-Biol., 16, 1.
6. **Mavor, J. W., and D. M. DeForest.**
'25 Proc. Exp. Biol. Med., XXII, 19.